Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL

CROSSLINKING REAGENTS

Applicant: David A. Schwartz Serial No. To be assigned

Filed: March 22, 2001

Our Docket No.: 37154-753

# FIGURE 1

hydrazine

semicarbazide

$$R \underset{O}{\overset{H}{\bigvee}}_{N} NH_{2}$$

hydrazide

thiosemicarbazide

thiocarbazide

$$\begin{array}{c|cccc}
R & O & H & H \\
N & I & I \\
N & I & N \\
H & H & O
\end{array}$$

$$\begin{array}{c|cccc}
N & N & N \\
N & N & N \\$$

$$R \searrow O \\ N \\ NH_2 \\ H$$

carbonic acid dihydrazine

hydrazine carboxylate

### HELLER EHRMAN WHITE & MCAULIFFE LLP

Sheet 2 of 9

Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL

CROSSLINKING REAGENTS

Applicant: David A. Schwartz

Serial No. To be assigned Filed: March 22, 2001

Our Docket No.: 37154-753

$$\mathbf{H} + \mathbf{A} \xrightarrow{\text{pH 4.7 or 7.4}} \mathbf{Protem A} \xrightarrow{\mathbf{h}} \mathbf{N} \xrightarrow{\mathbf{N}} \mathbf{N} \xrightarrow{\mathbf{N}} \mathbf{N}$$

HELLER EHRMAN WHITE & MCAULIFFE LLP
Sheet 3 of 9
Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL CROSSLINKING REAGENTS

Applicant: David A. Schwartz

Serial No. To be assigned Our Docket No.: 37154-753

Filed: March 22, 2001

HELLER EHRMAN WHITE & MCAULIFFE LLP
Sheet 4 of 9

Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL CROSSLINKING REAGENTS

Applicant: David A. Schwartz

Serial No. To be assigned Filed: March 22, 2001

Our Docket No.: 37154-753

## FIGURE 4

HO
$$N$$
 $NH_2$ 
 $NH_2$ 
 $NH_2$ 
 $NH_2$ 
 $NH_3$ 
 $NH_3$ 
 $NH_4$ 
 $NH_4$ 
 $NH_5$ 
 $NH_5$ 

#### HELLER EHRMAN WHITE & MCAULIFFE LLP

Sheet 5 of 9
HYDRAZINE-BASED AND C BASED BIFUNCTIONAL

Title: HYDRAZINE-BASED AND C I CROSSLINKING REAGENTS

Applicant: David A. Schwartz Serial No. To be assigned

Serial No. To be assigned Filed: March 22, 2001 Our Docket No.: 37154-753

## FIGURE 5

### Preparation of 5'-aromatic aldehyde-modified oligonucleotide:

#### Preparation of 5'-heteroaromatic hydrazine-modified oligonucleotide:

#### Conjugation of hydrazino-modified oligonucleotide to aldehyde-modified oligonucleotide

#### HELLER EHRMAN WHITE & MCAULIFFE LLP

Sheet 6 of 9

Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL

CROSSLINKING REAGENTS

Applicant: David A. Schwartz
Serial No. To be assigned

Our Docket No.: 37154-753

Filed: March 22, 2001

### FIGURE 6

$$OCN$$
 $OCN$ 
 $OCN$ 

2) acetone, MeOH

$$\begin{array}{c|c}
 & O \\
 & N \\
 & O \\$$

NHS, DCC, THF

$$\begin{array}{c|c}
 & O \\
 & N \\
 & O \\$$

HELLER EHRMAN WHITE & MCAULIFFE LLP
Sheet 7 of 9

Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL CROSSLINKING REAGENTS

Applicant: David A. Schwartz

Filed: March 22, 2001 Serial No. To be assigned

Our Docket No.: 37154-753

$$\begin{array}{c} \text{OEt} \\ \text{EtO} \\ \text{Si} \\ \text{EtO} \end{array} \begin{array}{c} \text{OEt} \\ \text{NH}_2 \end{array} + \begin{array}{c} \text{SuO} \\ \text{NH}_2 \end{array} \begin{array}{c} \text{THF} \\ \text{NH}_2 \end{array} \begin{array}{c} \text{THF} \\ \text{NH}_2 \end{array} \begin{array}{c} \text{NH}_2 \end{array} \begin{array}$$

### HELLER EHRMAN WHITE & MCAULIFFE LLP Sheet 8 of 9

HYDRAZINE-BASED AND C BASED BIFUNCTIONAL CROSSLINKING REAGENTS

Applicant: David A. Schwartz Serial No. To be assigned Our Docket No.: 37154-753

Filed: March 22, 2001

HELLER EHRMAN WHITE & MCAULIFFE LLP
Sheet 9 of 9
.itle: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL **CROSSLINKING REAGENTS** 

Applicant: David A. Schwartz

Serial No. To be assigned Filed: March 22, 2001

Our Docket No.: 37154-753